Increased risk of genetic and epigenetic instability in human embryonic stem cells associated with specific culture conditions.

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Public Summary:
We discovered that long term culture of human ESCs and iPSCs often results in selection for cells in which the TP53 gene is deleted. This work shows that low passage pluripotent cells should be used to ensure genomic integrity, and that culture of the cells on feeder layers is the best method for maintaining cells without introduction of mutations that would compromise their safety.

Scientific Abstract:
The self-renewal and differentiation capacities of human pluripotent stem cells (hPSCs) make them a promising source of material for cell transplantation therapy, drug development, and studies of cellular differentiation and development. However, the large numbers of cells necessary for many of these applications require extensive expansion of hPSC cultures, a process that has been associated with genetic and epigenetic alterations. We have performed a combinatorial study on both hESCs and hiPSCs to compare the effects of enzymatic vs. mechanical passaging, and feeder-free vs. mouse embryonic fibroblast feeder substrate, on the genetic and epigenetic stability and the phenotypic characteristics of hPSCs. In extensive experiments involving over 100 continuous passages, we observed that both enzymatic passaging and feeder-free culture were associated with genetic instability, higher rates of cell proliferation, and persistence of OCT4/POU5F1-positive cells in teratomas, with enzymatic passaging having the stronger effect. In all combinations of culture conditions except for mechanical passaging on feeder layers, we noted recurrent deletions in the genomic region containing the tumor suppressor gene TP53, which was associated with decreased mRNA expression of TP53, as well as alterations in the expression of several downstream genes consistent with a decrease in the activity of the TP53 pathway. Among the hESC cultures, we also observed culture-associated variations in global gene expression and DNA methylation. The effects of enzymatic passaging and feeder-free conditions were also observed in hiPSC cultures. Our results highlight the need for careful assessment of the effects of culture conditions on cells intended for clinical therapies.